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Caffeine Inhibits Human Immunodeficiency Virus Type 1 Transduction of Nondividing Cells

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Caffeine is an efficient inhibitor of DNA repair and DNA damage-activated checkpoints. We have shown recently that caffeine inhibits retroviral transduction of dividing cells, most likely by blocking postintegration repair. This effect may be mediated at least in part by a cellular target of caffeine, the ataxia telangiectasia-mutated and Rad3-related (ATR) kinase. In this study, we present evidence that caffeine also inhibits efficient transduction of nondividing cells. We observed reduced transduction in caffeine-treated growth-arrested cells as well as caffeine-treated terminally differentiated human neurons and macrophages. Furthermore, this deficiency was observed with a human immunodeficiency virus type 1 (HIV-1) vector lacking Vpr, indicating that the effect is independent of the presence of this viral protein in the infecting virion. Finally, we show that HIV-1 transduction of nocodazole-arrested cells is reduced in cells that express an ATR dominant-negative protein (kinase-dead ATR [ATRkd]) and that the residual transduction of ATRkd-expressing cells is relatively resistant to caffeine. Taken together, these data suggest that the effect(s) of caffeine on HIV-1 transduction is mediated at least partly by the inhibition of the ATR pathway but is not dependent on the caffeine-mediated inhibition of cell cycle checkpoints.

Cellular mechanisms that protect the integrity of chromosomal DNA are important for cell and organism survival. Surveillance mechanisms monitor the integrity of the genome; detection of DNA damage coordinately triggers checkpoint pathways and DNA repair systems (56). Activation of a DNA damage checkpoint results in cell cycle arrest, allowing time for DNA repair.

Caffeine belongs to a class of chemicals that strongly enhance the cytotoxic effect of ionizing radiation and other DNA-damaging agents, at concentrations that are not otherwise toxic to cells (3, 30, 53). The molecular mechanisms underlying this caffeine effect are still not fully understood. However, it has been established that caffeine disrupts DNA damage-activated cell cycle checkpoints. For example, it has been shown that caffeine eliminates p53 activation and G_1 arrest, G_2/M arrest, and S-phase delay in response to DNA damage (20, 23, 24, 26–28, 31, 34, 39, 42, 50–52). Nevertheless, it seems that not all caffeine effects are due to disruption of DNA damage checkpoints. It has been demonstrated that abrogation of a caffeine-mediated checkpoint does not correlate with the level of caffeine-induced radiosensitization (40). It is therefore likely that caffeine acts on both cell cycle checkpoints and directly on DNA repair.

DNA damage-activated cell cycle checkpoints are regulated by two related kinases, the ataxia telangiectasia-mutated (ATM) kinase and the ATM and Rad3-related (ATR) kinase, which belong to a family of phosphatidylinositol-3 kinase-related kinases (1, 46). ATM is activated primarily by double-strand DNA breaks, whereas ATR also responds to replication stress (1, 33, 46). While ATM and ATR activate cell cycle checkpoints in response to DNA damage, they also appear to play a direct role in DNA repair at sites of DNA damage (33). Caffeine disrupts ATM- and ATR-dependent checkpoint responses, possibly by direct inhibition of ATM and ATR kinase activities (21, 45, 55).

We recently observed that caffeine inhibits retroviral transduction of dividing cells (13). Transduction was also reduced in dividing cells that express a dominant-negative ATR protein, kinase-dead ATR [ATRkd], but not in cells that lack the related kinase, ATM (13). In the ATRkd-expressing cells, reduction in transduction efficiency was correlated with an integrase-dependent cell death. Because caffeine abrogates checkpoint responses to DNA damage and ATR is a major regulator of cell cycle checkpoints, one possible explanation of these data is that activation of cell cycle checkpoints is required for efficient retroviral DNA integration. However, we demonstrate in this study that caffeine also inhibits retroviral transduction of both drug-arrested and naturally nondividing cells. Furthermore, expression of ATRkd reduces transduction of nondividing cells. These data suggest that the effects of caffeine and the role of ATR in retroviral DNA integration are unlikely to be mediated by abrogation or activation, respectively, of cell cycle checkpoints. These findings lend further support to the hypothesis that ATR function is required for postintegration repair of the retroviral DNA integration intermediate in both dividing and nondividing cells.

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MATERIALS AND METHODS

Cells. Normal mouse embryonic fibroblasts (MEFs) were generously provided by the laboratory of M. Bosma (Fox Chase Cancer Center) and were maintained in RPMI 1640 medium in the presence of 10% fetal bovine serum, 5×10^{-6} M

2-mercaptoethanol, and penicillin-streptomycin. 293T cells and GM847/ATRkd cells were maintained in Dulbecco's modified Eagle medium with 10% fetal bovine serum and penicillin-streptomycin.

Isolation and culture of human primary macrophages. Human peripheral blood mononuclear cells were isolated by centrifugation in Ficoll-Hypaque (Sigma, St. Louis, Mo.) from buffy coats of human immunodeficiency virus type 1(HIV-1)-seronegative individuals (7). Monocyte-derived macrophages were obtained from peripheral blood mononuclear cells by adherence to plastic for 12 h in Dulbecco's modified Eagle medium supplemented with 10% human serum (Cellgro, Herndon, Va.), washed, and cultured in the same medium in the presence of macrophage colony-stimulating factor (2 ng/ml; Sigma, St. Louis, Mo.) for another 7 to 10 days, allowing cells to differentiate before infection (2). The medium was replaced twice during the incubation period. The primary cells were kept at 37°C in a humidified incubator with 5% CO_2.

hNT-2 cell cultures. Human NT-2 (hNT-2) neuronal precursor cells were purchased from Stratagene (La Jolla, Calif.) (Stratagene cloning system), cultured, and differentiated into mature human neurons (over 95%) after treatment with retinoic acid, as previously described (35–38). Mature neurons generated by differentiating hNT-2 cells were characterized by immunostaining for expression of ubiquitous neuronal markers (such as MAP2β and τ), as well as phenotypically elaborating extensive neuritic processes identifiable as axons and dendrites.

HIV-1-based vectors. The vesicular stomatitis virus G-pseudotyped HIV-1-based vector containing Vpr and carrying a *lacZ* reporter gene was prepared as described previously (13–16). HIV-1-based vectors carrying the D64V substitution in retroviral integrase or lacking *vpr*, *vif*, *vpu*, and *nef* genes were produced as above. Backbone plasmids for the multiply attenuated vector lacking *vpr*, *vif*, *vpu*, and *nef* genes or encoding the D64V mutation were obtained from D. Trono, University of Geneva (32, 57).

Cell cycle arrest. To arrest cells in M phase, cells were treated with nocodazole (1 μg/ml) for 24 h prior to addition of the virus. Nocodazole was maintained in the cell culture medium during and after infection, up to the time of staining for β-galactosidase activity. MEFs were arrested in G_1/G0 phase by contact inhibition. MEFs were distributed in a 96-well plate at a density of 2×10^4 cells per well. The following day, the MEFs reached a density of approximately 10^5 cells per well and were arrested in G_0/G_1 by contact inhibition, as shown by Western blot analysis.

Viral transductions. For studies with 293T cell cultures, cells were distributed in a 24-well plate at a density of 5×10^4 cells per well, and nocodazole was added to a final concentration of 1 μg/ml. Cells were infected 24 h later with the HIV-1-based vectors in the presence of 5 μg of DEAE dextran/ml. Caffeine was added to cells along with the vector and maintained on cells for 24 h. Two days postinfection, cells were stained by a β-galactosidase assay, and blue cells were counted. To control for a possible caffeine contamination, we also treated 293T cells with caffeine from different sources (Upstate; U.S. Biochemicals). The results obtained were consistent with those observed with caffeine from Sigma.

For infections of MEFs, cells were distributed in a 96-well plate at a density of 5×10^4 cells per well to prepare confluent cells or at a density of 1×10^4 cells per well to obtain exponentially growing cells. The following day, the cultures were infected with the HIV-1-based vectors in the presence of 5 μg of DEAE dextran/ml. Caffeine was added to cells at the same time as the vector and maintained in the medium for 24 h. Two days postinfection, cells were stained by a β-galactosidase assay, and blue cells were counted.

For studies with human macrophages and neurons, cells were prepared as described above. Caffeine was again added with the HIV-1-based vector in the presence of 5 μg of DEAE dextran/ml. Caffeine was maintained on the cells for 24 h (macrophages) or 48 h (neurons), and β-galactosidase staining was performed 2 days postinfection.

To infect ATRkd-expressing cells, GM847/ATRkd cells were plated at a density of 2×10^4 cells per well of a 24-well plate in the presence or absence of doxycycline (5 μg/ml) and nocodazole (1 μg/ml). The following day, cells were infected with the HIV-1-based vectors in the presence or absence of doxycycline and nocodazole; in the experiments described in Fig. 5, they were infected in the presence of caffeine. Doxycycline and caffeine were removed 24 h later, while nocodazole was maintained on the cells until 2 days postinfection, when the cultures were stained by the β-galactosidase assay.

Western blot analyses. For detection of PCNA (proliferating cell nuclear antigen) protein, cell lysates were resolved on sodium dodecyl sulfate–12% polyacrylamide gel electrophoresis gels, and Western blotting was performed with an anti-PCNA antibody (sc-56; Santa Cruz, Santa Cruz, Calif.). For detection of phosphorylated histone H3, the histone-containing fraction of cell lysates was heated to 90°C, sonicated, and resolved on sodium dodecyl sulfate–12% polyacrylamide gel electrophoresis gels. Western blotting was then performed with an anti-phosphorylated histone H3 (Ser 10) antibody (sc-8656-R; Santa Cruz).

RESULTS

Transduction of nocodazole-arrested 293T cells is sensitive to caffeine. We have shown previously that caffeine inhibits retrovirus transduction of exponentially growing HeLa cells, probably by affecting postintegration repair (13). Caffeine is a known inhibitor of DNA repair and is thought to exert its effects on this process predominantly through the inhibition of cell cycle checkpoint responses to DNA damage (see the introduction). It was conceivable, therefore, that the observed inhibition of retroviral transduction by caffeine may be limited to dividing cells.

To determine if caffeine has any effect on HIV-1 transduction of nondividing cells, we infected exponentially dividing and nocodazole-arrested 293T cells with an HIV-1-based vector (32). As shown in Fig. 1A and B, caffeine inhibited HIV-1 transduction of the dividing (A) and nocodazole-arrested (B) cells in the same dose-dependent manner. Similar results were obtained with HeLa cells (data not shown). Caffeine also inhibited transduction by a multiply attenuated HIV-1-based vector which lacked the *vpr*, *vif*, *vpu*, and *nef* genes (MAV; Fig. 1A and B) (57). No caffeine cytotoxicity was observed under these experimental conditions. To determine if the transduced *lacZ* gene was expressed from integrated vector DNA, we infected 293T cells with a vector carrying an inactivating D64V substitution in HIV-1 integrase (32). As shown in Fig. 1A and B, this vector transduced 293T cells with about a 10-fold-lower efficiency than the vector carrying wild-type integrase. We therefore conclude that the majority of reporter gene expression is derived from integrated proviral DNA.

To determine the efficiency of the nocodazole arrest, we assayed for expression of PCNA. PCNA accumulates in cells as they enter S phase but is rapidly degraded in other phases of the cell cycle (48). Figure 1C shows that the amount of PCNA in nocodazole-treated cells is only about 5% or less of that detected in exponentially dividing cells, indicating an efficient nocodazole-mediated growth arrest. Finally, we examined phosphorylation of histone H3 on serine 10, which is tightly associated with mitosis (11). Figure 1D reveals increased histone H3 phosphorylation on serine 10 of nocodazole-treated cells, consistent with nocodazole-mediated mitotic arrest. Finally, to determine if the observed HIV-1 transduction occurred in the few cells that still divided, nocodazole-arrested 293T cells were infected with a high-titer vector, which resulted in transduction of approximately of 25% of cells in the absence of caffeine. However, caffeine efficiently reduced HIV-1 transduction efficiency even under these conditions (data not shown). We conclude that caffeine inhibits HIV-1 transduction of nocodazole-arrested 293T cells.

Transduction of contact-inhibited MEFs is reduced by caffeine. Nocodazole inhibits cellular passage through the M phase (25). We investigated next whether caffeine reduces transduction of cells arrested in G_1 phase. However, agents that arrest cells in G_1/S, such as aphidicolin and hydroxyurea, also trigger an ATR-dependent DNA damage response (49). Therefore, in the following experiments, we used MEFs, which are very sensitive to contact inhibition (44). We observed that

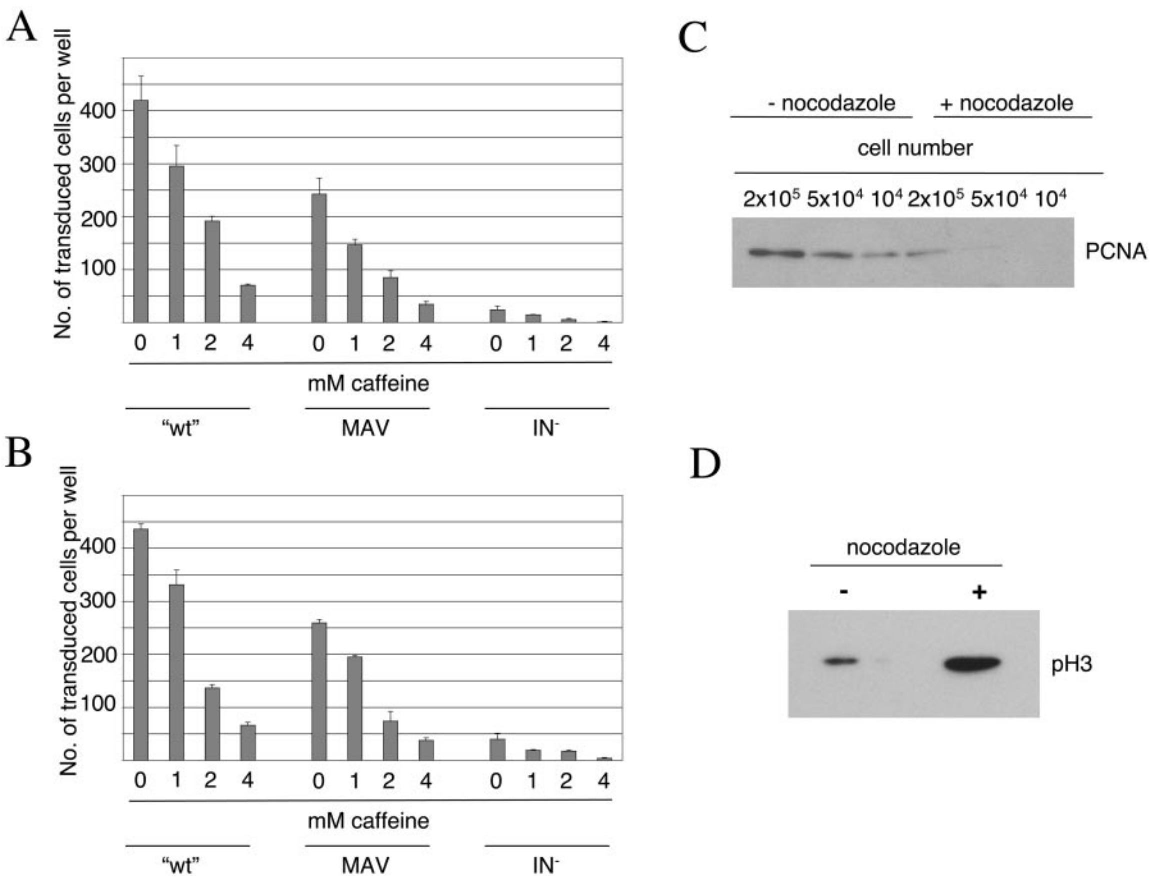

FIG. 1. Effect of caffeine on HIV-1 transduction of nocodazole-arrested cells. (A) Exponentially dividing 293T cells were infected with the same aliquots of HIV-1-based vectors and exposed to the indicated concentrations of caffeine for 24 h, as described in Materials and Methods. Two days postinfection, cells were stained by a β-galactosidase assay, and blue cells were counted. (B) 293T cells were infected and treated with caffeine as described in the legend to panel A, except they were arrested with nocodazole 24 h prior to addition of the vectors. (C) Amount of PCNA in dividing and nocodazole-treated 293T cells. Cells were treated with nocodazole as described in the legend to panel B for 24 h, at which time they were harvested and Western blot analysis was performed. (D) Ser 10-phosphorylated histone H3 in dividing and nocodazole-treated cells. "wt," HIV-1-based vector containing Vpr and wild-type integrase; MAV, multiply attenuated HIV-1-based vector; IN^-, HIV-1-based vector carrying a D64V substitution in retroviral integrase.

contact inhibition of MEFs led to a substantial reduction in the absolute numbers of HIV-1-transduced cells (Fig. 2A and B); cells were infected with the same amount of virus at the same time. However, transduction of both exponentially growing (A) and contact-inhibited (B) cells was further reduced by treatment with caffeine in a similar, dose-dependent manner. In each case, no caffeine-associated cytotoxicity was observed at the concentrations utilized. To confirm that contact inhibition of MEFs resulted in efficient growth arrest, we examined levels of PCNA protein and phosphorylation (Ser 10) of histone H3. Figure 2C shows that the amount of PCNA in contact-inhibited MEFs was only about 2% of that in exponentially dividing MEFs. A reduction in the amount of phosphorylated histone H3 was also observed (Fig. 2D), consistent with MEF growth arrest. We conclude that caffeine reduces transduction of arrested MEFs.

Transduction of terminally differentiated neuronal cells and macrophages is inhibited by caffeine. We next investigated the effect of caffeine on transduction of naturally arrested human cells. Terminally differentiated, postmitotic neurons were infected with the HIV-1 vector. As shown in Fig. 3A, caffeine also reduced the efficiency of transduction of these cells with the HIV-1-based vector. To verify that the cells were not cycling, we again measured the amount of PCNA protein. Figure 3B shows that PCNA expression was not detected in these differentiated neuronal cells. Lastly, we examined the effect of caffeine on transduction of terminally differentiated primary human macrophages. As with the neurons, we found that caffeine inhibits transduction of these cells, under conditions that showed no visible cytotoxicity (Fig. 3C).

Transduction of nocodazole-arrested cells is inhibited by expression of the dominant-negative, kinase-dead ATR, ATRkd. The ATR gene is an essential gene; its knockout phenotype is embryonically lethal in mice, and cultured cells die rapidly after the ATR gene is excised (4, 9, 17). However, cells that express a dominant-negative, kinase-dead ATR protein (GM847/ATRkd) are viable, although they have deficiencies in DNA repair and/or checkpoint regulation (6). In the cells used for these studies, the ATRkd gene was under control of a doxycycline-inducible promoter (6). As reported previously and shown in Fig. 4A, we observed a doxycycline-dependent reduction in the percentage of dividing cells that are trans-

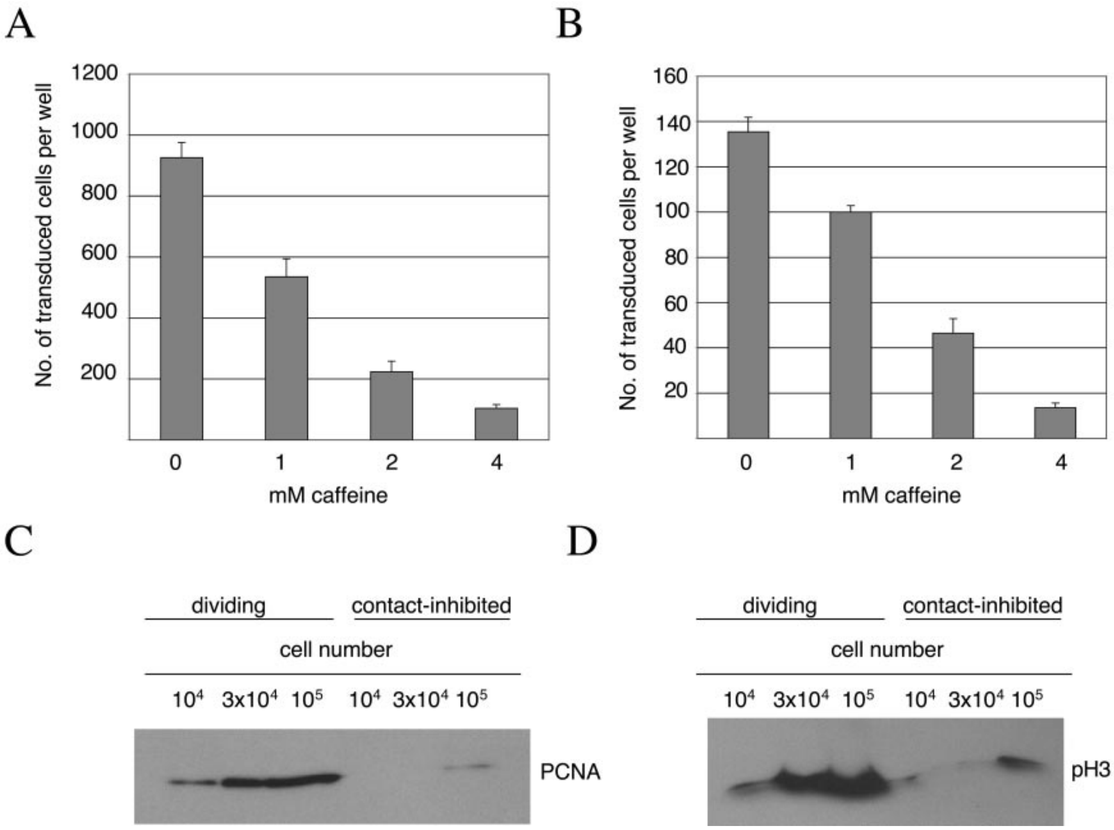

FIG. 2. Effect of caffeine on transduction of contact-inhibited MEFs. (A) Exponentially dividing MEFs were infected with the HIV-1 base vector carrying Vpr and exposed to caffeine for 24 h, as described in Materials and Methods. Two days postinfection, cells were stained by a β-galactosidase assay, and blue cells were counted. (B) MEFs were distributed in 96-well plates as described in the legend to panel A and infected at the point of confluency. Caffeine was added as described in the legend to panel A. (C) PCNA in dividing and confluent MEFs. Cells were treated as described in the legends to panel A and B, and Western blot analysis was performed at the time when MEFs would be infected. (D) Ser 10-phosphorylated histone H3 in dividing and confluent MEFs.

duced by the HIV-1-based vector (13). Doxycycline had no effect on the transduction of parental GM847 cells (data not shown). Because ATR was also implicated in the regulation of Vpr-induced G_2/M arrest (41), we also examined transduction of ATRkd-expressing cells by the multiply attenuated HIV-1-based vector (57). We again observed reduced transduction of cells expressing the dominant-negative ATRkd protein, similar to that observed with the Vpr-containing HIV-1 vector (Fig. 1A). To examine the role of ATR in growth-arrested cells, we treated the GM847/ATRkd cells with nocodazole in addition to doxycycline. As shown in Fig. 4B, nocodazole-treated, ATRkd-expressing cells were transduced with HIV-1-based vectors at a reduced level, when compared to control nocodazole-arrested cells. As was the case with 293T cells, Fig. 4C shows that the amount of PCNA in nocodazole-treated cells was only about 10 to 20% of that in exponentially dividing cells, indicating an efficient nocodazole-mediated growth arrest. Finally, Fig. 4D shows an increase in histone H3 phosphorylation on serine 10 in nocodazole-treated cells, consistent with the nocodazole-mediated mitotic arrest. We conclude that ATR function is required for efficient transduction of nocodazole-arrested cells.

Residual HIV-1 transduction of ATRkd-expressing cells is relatively resistant to caffeine. ATM and ATR kinases are reported to be two major cellular targets of caffeine and we have shown previously that the HIV-1 transduction of ATM-deficient cells is inhibited by caffeine with the same efficiency as transduction of ATM-proficient cells (13). To determine if the residual transduction of ATRkd-expressing cells can be inhibited by caffeine, we treated GM847/ATRkd cells with doxycycline, infected them with the HIV-1-based vector, and treated them with caffeine. As shown in Fig. 5, caffeine inhibited HIV-1 transduction of GM847/ATRkd cells in the absence of doxycycline as efficiently as it inhibited transduction of 293T cells (Fig. 1). In the presence of doxycycline, the effect of caffeine was markedly different. Addition of 0.5 mM caffeine led to a 40% drop in transduction efficiency, regardless of the presence or absence of doxycycline. However, further increase in caffeine concentrations had little effect on transduction efficiency of doxycycline-treated, ATRkd-expressing cells. At the highest caffeine concentration, 4 mM, the transduction efficiency of doxycycline-treated cells was reduced only twofold when compared to control cells infected in the absence of caffeine. In contrast, addition of 4 mM caffeine led to a ninefold reduction in transduction efficiency of GM847/ATRkd cells infected in the absence of doxycycline. We conclude that that HIV-1 transduction of ATRkd-expressing cells is rela-

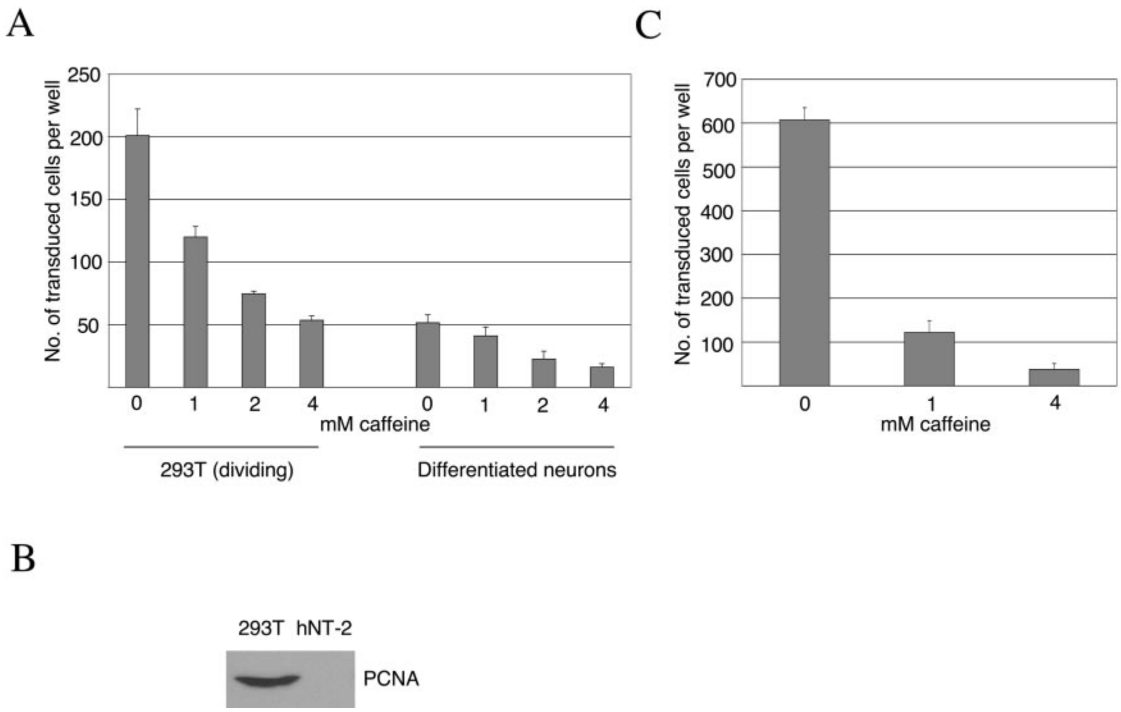

FIG. 3. Effect of caffeine on transduction of terminally differentiated neurons and macrophages. (A) Terminally differentiated hNT-2 neurons were prepared as described in Materials and Methods, infected with the HIV-1-based vector carrying Vpr, and exposed to caffeine for 24 h. Two days postinfection, cells were stained by a β-galactosidase assay, and blue cells were counted. (B) PCNA in terminally differentiated neurons. Cells were treated as described in the legend to panel A, and Western blot analysis was performed at the time when cells would be infected, with 2×10^5 cells per lane. (C) Effect of caffeine on transduction of terminally differentiated macrophages. Terminally differentiated macrophages were prepared as described in Materials and Methods, infected with the HIV-1-based vector, and exposed to caffeine for 24 h. Two days postinfection, cells were stained by a β-galactosidase assay, and blue cells were counted.

tively resistant to caffeine. These data suggest that the effect of caffeine may be mediated by inhibition of the ATR pathway.

DISCUSSION

We reported recently that retroviral transduction can be blocked by caffeine, an inhibitor of host cell DNA repair. We have also shown that efficient retroviral transduction requires the cellular ATR protein, which is a known caffeine target. However, only cycling cells were used in experiments described in the initial work (13). Because nondividing cells, such as macrophages, are also main targets of HIV-1 infection and caffeine is presumed to exert its effect on DNA repair primarily by regulation of cell cycle checkpoints, it could be expected that caffeine will affect retroviral transduction only in cycling cells. The studies described here show that caffeine also inhibits HIV-1 transduction of nondividing cells.

We performed our initial experiments with nocodazole-arrested 293T cells in M phase and observed that caffeine inhibits the HIV-1 transduction in nondividing 293T cells with the same efficiency as in dividing 293T cells. It has been reported that in nondividing cells, unintegrated HIV-1-based vector DNA may be expressed as efficiently as integrated DNA (43). Therefore, we also infected the growth-arrested cells with a control vector carrying an inactivating D64V substitution in HIV-1 integrase (32). We observed that the transduction efficiency of this vector is about 10-fold lower than that of the vector with a wild-type integrase gene. Therefore, we conclude that the majority of the observed expression is from integrated proviral DNA.

To determine if caffeine inhibits HIV-1 transduction of cells arrested in G_1/G_0 phase, we examined contact-inhibited MEFs. As with the nocodazole-arrested 293T cells, we observed that caffeine also reduces transduction of the contact-inhibited MEFs. The majority of the reporter gene expression in these cells was again derived from integrated proviral DNA, as described previously (12). Caffeine treatment also inhibited HIV-1 transduction of terminally differentiated, postmitotic neurons and macrophages.

Caffeine inhibits checkpoint activation in response to DNA damage, a process that is known to be regulated by the ATM and ATR kinases (1, 46). Caffeine was found to inhibit catalytic activities of these kinases in vitro, at concentrations that are required to induce radiosensitization in vivo (21, 45). In vivo, caffeine reduces ATM-mediated Chk2/Cds1 activation and phosphorylation (55). Therefore, it has been assumed that caffeine exerts its effects by direct inhibition of ATM and ATR kinases, and this drug has been used widely to study the function of ATM and ATR in cultured cells (10, 18, 22, 29, 47, 54). It was reported very recently that the phosphorylation of some ATM and ATR substrates in cultured cells is not inhibited by caffeine (8). However, subsequent published results indicate that caffeine does inhibit the ATR and ATM kinases in vivo (5, 19). As we had shown that ATM-deficient cells are transduced

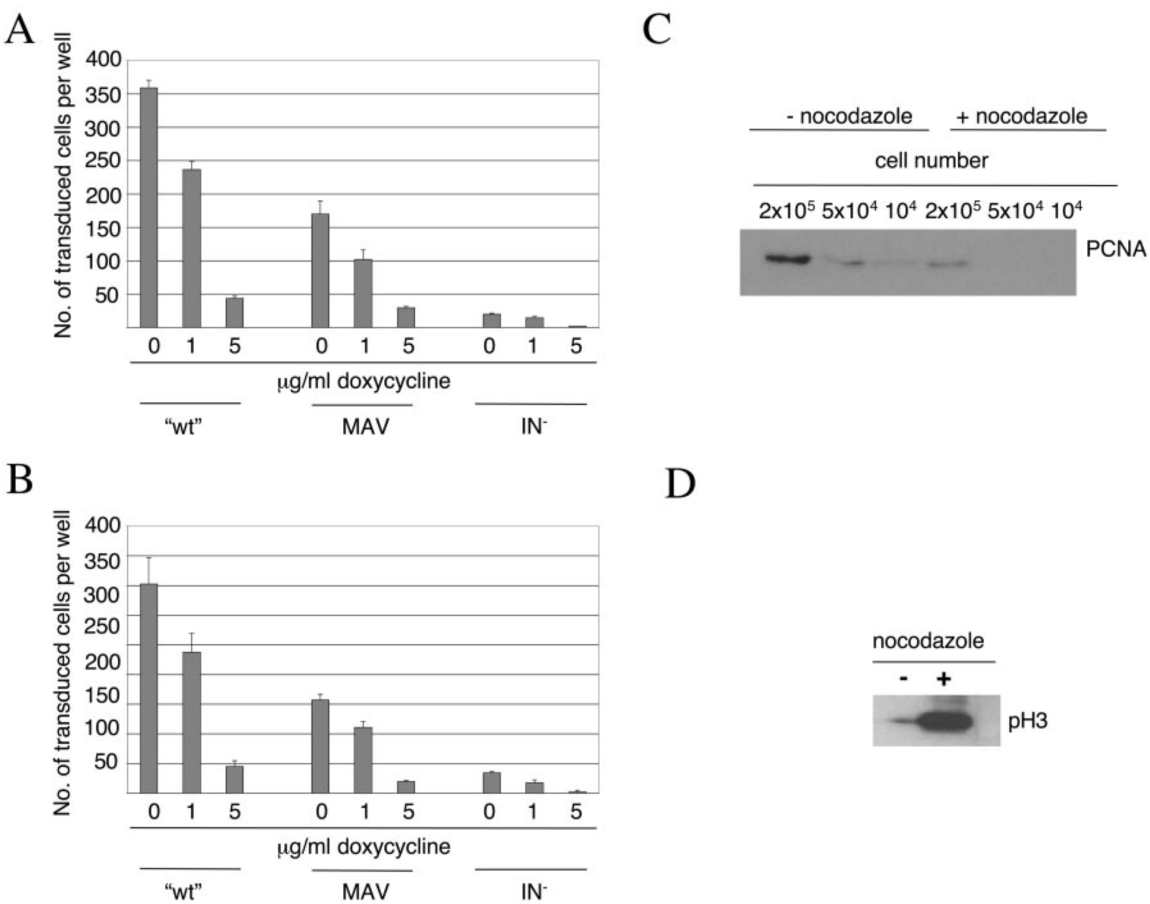

FIG. 4. Effect of overexpression of dominant-negative, kinase-dead ATRkd protein on transduction of nocodazole-arrested cells. (A) Exponentially dividing GM847/ATRkd cells were exposed to doxycycline and infected with the HIV-1-based vectors as described in Materials and Methods. Two days postinfection, cells were stained by a β-galactosidase assay, and blue cells were counted. (B) GM847/ATRkd cells were infected and doxycycline treated as described in the legend to panel A, except they were growth arrested with nocodazole 24 h prior to addition of the viruses. (C) PCNA in dividing and nocodazole-treated GM847/ATRkd cells. Cells were treated as described in the legend to panel A, and Western blot analysis was performed at the time when cells would be infected. (D) Ser 10-phosphorylated histone H3 in dividing and nocodazole-treated GM847/ATRkd cells. "wt," HIV-1-based vector containing Vpr protein and wild-type integrase; MAV, multiply attenuated HIV-1-based vector; IN^-, HIV-1-based vector carrying a D64V substitution in retroviral integrase.

at a normal efficiency (13, 14), it seemed likely that the observed effect of caffeine on HIV-1 infection is mediated by its inhibition of the ATR kinase.

We examined retroviral transduction of nacodazole-arrested cells that express the dominant-negative ATRkd (6). Our results demonstrated that HIV-1 transduction of nondividing cells is reduced upon expression of ATRkd in a manner similar to that observed with dividing cells.

As it has been reported that the Vpr protein may trigger the cellular ATR-dependent DNA damage response, we also included an HIV-1-based vector lacking the Vpr gene in our experiments (41, 57). However, transduction efficiency of this vector was as sensitive to the expression of the ATRkd transdominant mutant as that of the Vpr-carrying HIV-1-based vector (Fig. 4A). These data suggest that ATR may play at least a dual role in the HIV-1 life cycle, affecting both Vpr-induced growth arrest and retroviral DNA integration.

Finally, to determine if the effect of caffeine on HIV-1 transduction is due to inhibition of the ATR pathway, we treated cells expressing the dominant-negative ATRkd protein with caffeine. We observed that the residual HIV-1 transduction of ATRkd-expressing cells is less sensitive to caffeine treatment than transduction of ATR-proficient cells. These results suggest that the caffeine effect on HIV-1 transduction is at least partly mediated by inhibition of the ATR pathway.

We proposed previously that one possible explanation for the effects of caffeine and ATRkd expression on retroviral transduction is that the retroviral DNA integration intermediate elicits a DNA damage response, which in turn leads to a transient cell cycle arrest that allows time for cellular DNA repair enzymes to complete the required postintegration repair reactions (13). Our finding that HIV-1 transduction of nondividing cells is also reduced by caffeine and expression of ATRkd argues against a requirement for checkpoint activation. Rather, the studies reported here support the alternative explanation, namely that ATR is directly involved in postintegration repair at sites of retroviral DNA integration, through either recruitment or modification of the necessary repair proteins.

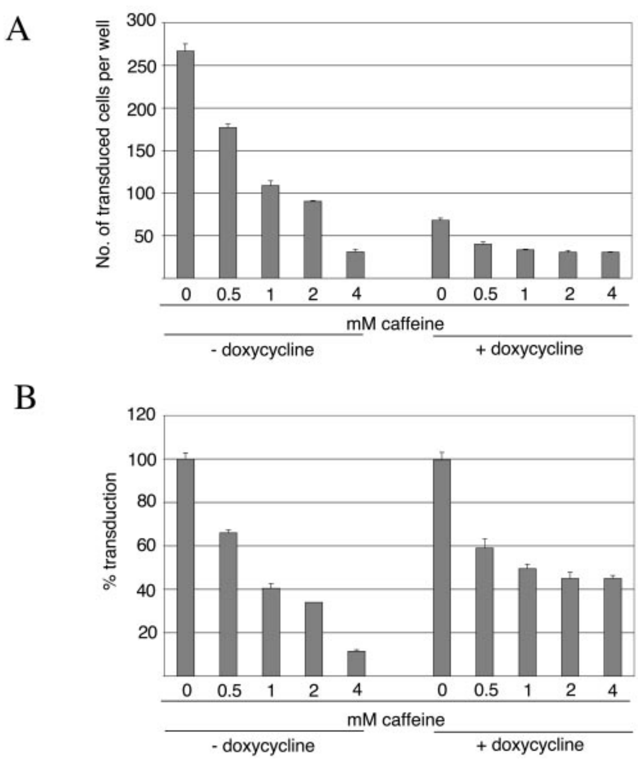

FIG. 5. Effect of caffeine on HIV-1 transduction of ATR-deficient cells. (A) Exponentially dividing GM847/ATRkd cells were exposed to doxycycline (5 μg/ml), infected with the same aliquots of HIV-1-based vectors, and treated with the indicated concentrations of caffeine as described in Materials and Methods. Two days postinfection, cells were stained by a β-galactosidase assay, and transduced cells were counted. (B) Caffeine effect expressed as relative transduction efficiency. A 100% value indicates the number of transduced cells in absence of caffeine, irrespective of the presence or absence of doxycycline.

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